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(54) Title: IMMUNISING AGAINST MENINGOCOCCAL SEROGROUP Y USING PROTEINS

(57) Abstract: The established dogma for meningococcus is thus that immunisation against serogroups A, C, W135 and Y shall be based on the four different capsular saccharides, and that immunisation against serogroup B shall not be based on the capsular saccharide. In contrast, the invention uses polypeptide antigens and/or OMVs to immunise against serogroups A, C, W135 and Y (and against serogroup Y in particular). Serogroup B polypeptides can achieve this protection, thus permitting a single polypeptide-based vaccine to be used for protecting against all of serogroups A, B, C, W 135 and Y.

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IMMUNISING AGAINST MENINGOCOCCAL SEROGROUP Y USING PROTEINS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens from *Neisseria meningitidis* (meningococcus) and their use in immunisation.

BACKGROUND ART

N.meningitidis is a non-motile, Gram-negative human pathogen that colonises the pharynx and causes meningitis (and, occasionally, septicaemia in the absence of meningitis). It causes both endemic and epidemic disease. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N.meningitidis* is the major cause of bacterial meningitis in the USA.

Based on the organism's capsular polysaccharide, various serogroups of *N.meningitidis* have been identified. Serogroup A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the USA and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the USA and developed countries. After serogroup, classification includes serotype, serosubtype and then immunotype, and the standard nomenclature lists serogroup, serotype, serosubtype, and immunotype, each separated by a colon *e.g.* B:4:P1.15:L3,7,9. Within serogroup B, some lineages cause disease often (hyperinvasive), some lineages cause more severe forms of disease than others (hypervirulent), and others rarely cause disease at all. Seven hypervirulent lineages are recognised, namely subgroups I, III and IV-1, ET-5 complex, ET-37 complex, A4 cluster and lineage 3. These have been defined by multilocus enzyme electrophoresis (MLEE), but multilocus sequence typing (MLST) has also been used to classify meningococci [ref. 1].

To date, vaccines against serogroup A, C, W135 and Y have used their capsular saccharides as antigens. A licensed human polysaccharide vaccine against these four serogroups has been known for many years [2,3]. More recently the focus has remained on saccharides, but the conjugation to carrier proteins. Conjugate vaccines against serogroup C have been approved for human use, and include Menjugate™ [4], Meningitec™ and NeisVac-C™. Mixtures of conjugates from serogroups A+C are known [5,6] and from serogroups A+C+W135+Y have been reported [7-10].

The capsular saccharide of serogroup B cannot be used for vaccination because it is a self-antigen in humans. Chemically-modified serogroup B saccharides have been proposed [11] but have not been adopted for clinical use. Vaccines based on outer-membrane vesicles have also been tested [*e.g.* see ref. 34], but the protection afforded by these vaccines is typically restricted to the strain used to make the vaccine. Genome sequences for serogroups A [12] and B [13,14] have been reported, and the serogroup B sequence has been studied to identify vaccine antigens [*e.g.* refs. 15 to 20]. Candidate antigens have been manipulated to improve heterologous expression [refs. 21 to 23].

The established dogma for meningococcus is thus that immunisation against serogroups A, C, W135 and Y shall be based on the four different capsular saccharides, and that immunisation against serogroup B shall not be based on the capsular saccharide.

DISCLOSURE OF THE INVENTION

In contrast to this dogma, the inventors have found that immunisation against serogroups A, C, W135 and Y (and against serogroup Y in particular) can be achieved using polypeptide antigens. Moreover, they have found that serogroup B polypeptides can achieve this protection, thus permitting a single polypeptide-based vaccine to be used for protecting against all of serogroups A, B, C, W135 and Y.

Thus the invention provides a method of immunising a subject against infection by serogroup Y of *Neisseria meningitidis*, comprising administering to the subject a composition comprising one or more immunogenic polypeptides. Similarly, the invention provides the use of one or more immunogenic polypeptides in the manufacture of a medicament for immunising a subject against infection by serogroup Y of *N.meningitidis*.

The invention also provides a method of immunising a subject against infection by serogroup Y of *Neisseria meningitidis*, comprising administering to the subject a composition comprising meningococcal OMVs. Similarly, the invention provides the use of meningococcal OMVs in the manufacture of a medicament for immunising a subject against infection by serogroup Y of *N.meningitidis*.

The methods and uses are preferably for immunising a subject against infection by serogroup Y and also against at least one of serogroups A, B, C and W135. Where a subject is being immunised against a given serogroup of meningococcus then the composition preferably does not include a capsular saccharide from that serogroup (either conjugated or non-conjugated). Thus preferred compositions do not include a capsular saccharide from serogroup Y, and may also not include a capsular saccharide from serogroups A, B, C and/or W135.

Compositions for use according to the invention can be prepared using known techniques, such as the techniques for preparing meningococcal polypeptide antigens disclosed in references 15-24, or the known techniques for preparing OMVs disclosed in references 34-38. The use of purified polypeptide antigens is preferred to the use of outer membrane vesicles.

Vaccines against pathogens such as hepatitis B virus, diphtheria and tetanus typically contain a single protein antigen (*e.g.* the HBV surface antigen, or a tetanus toxoid). In contrast, acellular whooping cough vaccines typically contain at least three *B.pertussis* proteins and the Prevenar™ pneumococcal vaccine contains seven separate conjugated saccharide antigens. Other vaccines such as cellular pertussis vaccines, the measles vaccine, the inactivated polio vaccine (IPV) and meningococcal OMV vaccines are by their very nature complex mixtures of a large number of antigens. Whether

protection can be elicited by a single antigen, a small number of defined antigens, or a complex mixture of undefined antigens, therefore depends on a number of factors.

The immunogenic polypeptide(s)

In some embodiments, the invention involves administration of at least one immunogenic polypeptide to subjects in order to provide protection against *Neisseria meningitidis* infection. These immunogenic polypeptides will generally include meningococcal amino acid sequences, such as amino acid sequences found in serogroup B strains, such as the sequenced MC58 strain [13].

A small number of defined antigens may be used. Rather than consisting of a single antigen, therefore, it is preferred that the composition of the invention comprises a mixture of 10 or fewer (*e.g.* 9, 8, 7, 6, 5, 4, 3, 2) purified antigens, and it is particularly preferred that the composition should not include complex or undefined mixtures of antigens *e.g.* it is preferred not to include outer membrane vesicles in the composition.

Preferred immunogenic polypeptides for use with the invention are those disclosed in reference 24: (1) a 'NadA' protein; (2) a '741' protein; (3) a '936' protein; (4) a '953' protein; and (5) a '287' protein. These antigens are referred to herein as the 'five basic antigens'. The invention may use 1, 2, 3, 4 or all 5 of these antigens.

NadA protein

'NadA' (Neisserial adhesin A) from serogroup B of *N.meningitidis* is disclosed as protein '961' in reference 17 (SEQ IDs 2943 & 2944) and as 'NMB1994' in reference 13 (see also GenBank accession numbers: 11352904 & 7227256). A detailed description of the protein can be found in reference 25. No corresponding protein was seen in the serogroup A genome [12, 25], but $NadA^{+}$ serogroup A strains have been reported since [25].

When used according to the present invention, NadA may take various forms. Preferred forms of NadA are truncation or deletion variants, such as those disclosed in references 21 to 23. In particular, NadA without its C-terminal membrane anchor is preferred (*e.g.* deletion of residues 351-405 for strain 2996 [SEQ ID NO: 1]), which is sometimes distinguished herein by the use of a 'C' superscript *e.g.* $NadA^{(C)}$. Expression of NadA without its membrane anchor domain (*e.g.* SEQ ID NO: 1) in *E.coli* results in secretion of the protein into the culture supernatant with concomitant removal of its 23mer leader peptide (*e.g.* to leave a 327mer for strain 2996 [SEQ ID NO: 2]). Polypeptides without their leader peptides are sometimes distinguished herein by the use of a 'NL' superscript *e.g.* $NadA^{(NL)}$ or $NadA^{(C)(NL)}$.

Preferred NadA sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 2. This includes NadA variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of NadA are shown in Figure 9 of reference 26.

Other preferred NadA sequences comprise at least *n* consecutive amino acids from SEQ ID NO: 1, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from NadA. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID NO: 1 (*e.g.* $NadA^{(C)}$, $NadA^{(NL)}$, $NadA^{(C)(NL)}$). Where N-terminus residues are deleted, it is preferred that the deletion should not remove the ability of NadA to adhere to human epithelial cells. A preferred fragment of SEQ ID NO: 1 is SEQ ID NO: 2.

NadA is preferably used in an oligomeric form (*e.g.* in trimeric form).

741 protein

'741' protein from serogroup B is disclosed in reference 17 (SEQ IDs 2535 & 2536) and as 'NMB1870' in reference 13 (see also GenBank accession number GI:7227128). The corresponding protein in serogroup A [12] has GenBank accession number 7379322. 741 is naturally a lipoprotein.

When used according to the present invention, 741 protein may take various forms. Preferred forms of 741 are truncation or deletion variants, such as those disclosed in references 21 to 23. In particular, the N-terminus of 741 may be deleted up to and including its poly-glycine sequence (*i.e.* deletion of residues 1 to 72 for strain MC58 [SEQ ID NO: 3]), which is sometimes distinguished herein by the use of a 'ΔG' prefix. This deletion can enhance expression. The deletion also removes 741's lipidation site.

Preferred 741 sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 3. This includes 741 variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of 741 can be found in SEQ IDs 1 to 22 of reference 23, and in SEQ IDs 1 to 23 and 123-141 of reference 27. SEQ IDs 1-299 of reference 28 give further 741 sequences.

Other preferred 741 sequences comprise at least *n* consecutive amino acids from SEQ ID NO: 3, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 741. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID NO: 3.

Protein 741 is an extremely effective antigen for eliciting anti-meningococcal antibody responses, and it is expressed across all meningococcal serogroups. Phylogenetic analysis shows that the protein splits into two groups, and that one of these splits again to give three variants in total [29], and while serum raised against a given variant is bactericidal within the same variant group, it is not active against strains which express one of the other two variants *i.e.* there is intra-variant cross-protection, but not inter-variant cross-protection. For maximum cross-strain efficacy, therefore, it is preferred that a composition should include more than one variant of protein 741. An exemplary sequence from each variant is given in SEQ ID NO[S]: 10, 11 and 12 herein, starting with a N-terminal cysteine residue to which a lipid will be covalently attached in the lipoprotein form of 741.

It is therefore preferred that the composition should include at least two of: (1) a first protein, comprising an amino acid sequence having at least *a%* sequence identity to SEQ ID NO: 10 and/or comprising an amino acid sequence consisting of a fragment of at least *x* contiguous amino acids from SEQ ID NO: 10; (2) a second protein, comprising an amino acid sequence having at least *b%* sequence identity to SEQ ID NO: 11 and/or comprising an amino acid sequence consisting of a fragment of at least *y* contiguous amino acids from SEQ ID NO: 11; and (3) a third protein, comprising an amino acid sequence having at least *c%* sequence identity to SEQ ID NO: 12 and/or comprising an amino acid sequence consisting of a fragment of at least *z* contiguous amino acids from SEQ ID NO: 12.

The value of *a* is at least 85 *e.g.* 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, or more. The value of *b* is at least 85 *e.g.* 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, or more. The value of *c* is at least 85 *e.g.* 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, or more. The values of *a, b* and *c* are not intrinsically related to each other.

The value of *x* is at least 7 *e.g.* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 225, 250). The value of *y* is at least 7 *e.g.* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 225, 250). The value of *z* is at least 7 *e.g.* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 225, 250). The values of *x, y* and *z* are not intrinsically related to each other.

It is preferred that any given 741 amino acid sequence will not fall into more than one of categories (1), (2) and (3). Any given 741 sequence will thus fall into only one of categories (1), (2) and (3). It is thus preferred that: protein (1) has less than *i%* sequence identity to protein (2); protein (1) has less than *j%* sequence identity to protein (3); and protein (2) has less than *k%* sequence identity to protein (3). The value of *i* is 60 or more (*e.g.* 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, *etc.*) and is at most *a*. The value of *j* is 60 or more (*e.g.* 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, *etc.*) and is at most *b*. The value of *k* is 60 or more (*e.g.* 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, *etc.*) and is at most *c*. The values of *i, j* and *k* are not intrinsically related to each other.

936 protein

'936' protein from serogroup B is disclosed in reference 17 (SEQ IDs 2883 & 2884) and as 'NMB2091' in reference 13 (see also GenBank accession number GI:7227353). The corresponding gene in serogroup A [12] has GenBank accession number 7379093.

When used according to the present invention, 936 protein may take various forms. Preferred forms of 936 are truncation or deletion variants, such as those disclosed in references 21 to 23. In particular,

the N-terminus leader peptide of 936 may be deleted (*i.e.* deletion of residues 1 to 23 for strain MC58 [SEQ ID NO: 4]) to give $936^{(NL)}$.

Preferred 936 sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 4. This includes variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants *etc*). Other preferred 936 sequences comprise at least *n* consecutive amino acids from SEQ ID NO: 4, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 936. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID NO: 4.

953 protein

'953' protein from serogroup B is disclosed in reference 17 (SEQ IDs 2917 & 2918) and as 'NMB1030' in reference 13 (see also GenBank accession number GI:7226269). The corresponding protein in serogroup A [12] has GenBank accession number 7380108.

When used according to the present invention, 953 protein may take various forms. Preferred forms of 953 are truncation or deletion variants, such as those disclosed in references 21 to 23. In particular, the N-terminus leader peptide of 953 may be deleted (*i.e.* deletion of residues 1 to 19 for strain MC58 [SEQ ID NO: 5]) to give $953^{(NL)}$.

Preferred 953 sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 5. This includes 953 variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of 953 can be seen in Figure 19 of reference 19.

Other preferred 953 sequences comprise at least *n* consecutive amino acids from SEQ ID NO: 5, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 953. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID NO: 5.

287 protein

'287' protein from serogroup B is disclosed in reference 17 (SEQ IDs 3103 & 3104), as 'NMB2132' in reference 13, and as 'GNA2132' in reference 20 (see also GenBank accession number GI:7227388). The corresponding protein in serogroup A [12] has GenBank accession number 7379057.

When used according to the present invention, 287 protein may take various forms. Preferred forms of 287 are truncation or deletion variants, such as those disclosed in references 21 to 23. In particular, the N-terminus of 287 may be deleted up to and including its poly-glycine sequence (*i.e.* deletion of residues 1 to 24 for strain MC58 [SEQ ID NO: 6]), which is sometimes distinguished herein by the use of a 'ΔG' prefix. This deletion can enhance expression.

Preferred 287 sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 6. This includes 287 variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of 287 can be seen in Figures 5 and 15 of reference 19, and in example 13 and figure 21 of reference 17 (SEQ IDs 3179 to 3184).

Other preferred 287 sequences comprise at least *n* consecutive amino acids from SEQ ID 6, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 287. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID NO: 6.

Fusion proteins

The five antigens may be present in the composition as five separate polypeptides, but it is preferred that at least two of the antigens are expressed as a single polypeptide chain (a 'hybrid' protein [refs. 21 to 24]) *e.g.* such that the five antigens form fewer than five polypeptides. Hybrid proteins offer two principal advantages: first, a protein that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two separately-useful proteins.

A hybrid protein included in a composition of the invention may comprise two or more (*i.e.* 2, 3, 4 or 5) of the five basic antigens. Hybrids consisting of two of the five basic antigens are preferred.

Within the combination of five basic antigens, an antigen may be present in more than one hybrid protein and/or as a non-hybrid protein. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both, although it may be useful to include protein 741 both as a hybrid and a non-hybrid (preferably lipoprotein) antigen, particularly where more than one variant of 741 is used.

Two-antigen hybrids for use in the invention comprise: NadA & 741; NadA & 936; NadA & 953; NadA & 287; 741 & 936; 741 & 953; 741 & 287; 936 & 953; 936 & 287; 953 & 287. Preferred two-antigen hybrids comprise: 741 & 936; 953 & 287. See further details in reference 24.

Hybrid proteins can be represented by the formula $\mathbf{NH_2\text{-}A\text{-}[\text{-}X\text{-}L\text{-}]_n\text{-}B\text{-}COOH}$, wherein: X is an amino acid sequence of one of the five basic antigens; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and *n* is 2, 3, 4 or 5.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be

retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

For each *n* instances of [-X-L-], linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_n where n = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID NO: 9), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(Gly)_4$ tetrapeptide being a typical poly-glycine linker. If X_{n+1} is a ΔG protein and L_n is a glycine linker, this may be equivalent to X_{n+1} not being a ΔG protein and L_n being absent.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Most preferably, *n* is 2. Two preferred proteins of this type are: X_1 is a 936 and X_2 is a 741; X_1 is a 287 and X_2 is a 953.

Two particularly preferred hybrid proteins of the invention are as follows:

n	A	X_1	L_1	X_2	L_2	B	[SEQ ID NO:]
2	MA	ΔG287	SEQ ID NO: 9	$953^{(NL)}$	—	—	7
2	M	$936^{(NL)}$	SEQ ID NO: 9	ΔG741	—	—	8

These two proteins may be used in combination with NadA (particularly with SEQ ID NO: 2) [24].

Mixtures excluding OMVs and/or excluding lipooligosaccharide are preferred.

Outer membrane vesicles

As an alternative to using purified polypeptide antigens, the invention may employ preparations of *N.meningitidis* microvesicles [30], 'native OMVs' [31], blebs or outer membrane vesicles [*e.g.* refs. 32 to 37 *etc.*]. All of these various preparations are referred to herein under the general term 'OMVs'.

In some embodiments, OMVs may be prepared from bacteria that have been genetically manipulated [38-41] *e.g.* to increase immunogenicity (*e.g.* hyper-express immunogens), to reduce toxicity, to inhibit capsular polysaccharide synthesis, to down-regulate or knockout PorA expression, to down-regulate or knockout *lgtB* expression [42], *etc.* They may be prepared from hyperblebbing strains [43-46]. Vesicles from a non-pathogenic *Neisseria* may be included [47]. OMVs may be prepared without the use of detergents [48,49]. They may express non-Neisserial proteins on their surface [50]. They may be LPS-depleted. They may retain lipooligosaccharide as an important antigen [42,51]. They may be mixed with recombinant antigens [32,52]. They may be treated to reduce phase variability of lipooligosaccharide immunotype [53]. Mixtures of OMVs may be used [30] including mixtures from different serotypes and/or serosubtypes [30,54].

Vesicles from bacteria with different class I outer membrane protein subtypes may be used *e.g.* six different subtypes [55,56] using two different genetically-engineered vesicle populations each displaying three subtypes, or nine different subtypes using three different genetically-engineered vesicle populations each displaying three subtypes, *etc.* Useful subtypes include: P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13; P1.7-2,4; P1.22,14; P1.7-1,1; P1.18-1,3,6.

Result of immunisation

The result of the immunisation will be generation of antibodies in the subject that (a) recognise the immunogenic polypeptide and (b) are protective against infection by multiple meningococcal serotypes. A typical result of immunisation will be the generation of an antibody response that is bactericidal against at least serogroup Y meningococcus, and more typically against each of serogroups A, B, C, W135 and Y.

A preferred result is effective immunisation against: (a) serogroups Y and A; (b) serogroups Y and B; (c) serogroups Y and C; (d) serogroups Y and W135; *etc.* Immunisation against at least serogroups A, B, C and Y is preferred. Protection may also be provided against other (non-pathogenic) serogroups *e.g.* H, I, K, L, X, Z, 29E, *etc.* Protection may also be provided against other *Neisseria* species *e.g.* lactamica, gonorrhoeae, cinerea, *etc.*

After immunisation, a serum preferably has a bactericidal titre of at least 1024 (*e.g.* 2^{10}, 2^{11}, 2^{12}, 2^{13}, 2^{14}, 2^{15}, 2^{16}, 2^{17}, 2^{18} or higher, preferably at least 2^{14}) *i.e.* the serum is able to kill at least 50% of test bacteria of a particular strain when diluted 1/1024, as described in reference 20.

Serogroups and strains

The methods and uses of the invention are preferably for immunising a subject against infection by serogroup Y and also against at least one of serogroups A, B, C and W135.

Preferred proteins of the invention comprise an amino acid sequence found in *N.meningitidis* serogroup B. Within serogroup B, preferred strains are 2996, MC58, 95N477, and 394/98. Strain 394/98 is sometimes referred to herein as 'NZ', as it is a New Zealand strain.

Protein 287 is preferably from strain 2996 or, more preferably, from strain 394/98.

Protein 741 is preferably from serogroup B strains MC58, 2996, 394/98, or 95N477, or from serogroup C strain 90/18311. Strain MC58 is more preferred.

Proteins 936, 953 and NadA are preferably from strain 2996.

Strains may be indicated as a subscript *e.g.* 741_{MC58} is protein 741 from strain MC58. Unless otherwise stated, proteins mentioned herein (*e.g.* with no subscript) are from *N.meningitidis* strain 2996, which can be taken as a 'reference' strain. It will be appreciated, however, that the invention is not in general limited by strain. As mentioned above, general references to a protein (*e.g.* '287', '919' *etc.*) may be taken to include that protein from any strain. This will typically have sequence identity to 2996 of 90% or more (*eg.* 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more).

Where a composition includes a particular protein antigen (*e.g.* 741 or 287), the composition can include that antigen in more than one variant form *e.g.* the same protein, but from more than one strain. These proteins may be included as tandem or separate proteins.

Where hybrid proteins are used, the individual antigens within the hybrid (*i.e.* individual -X- moieties) may be from one or more strains. Where n=2, for instance, X_2 may be from the same strain as X_1 or from a different strain. Where n=3, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2\neq X_3$ (iii) $X_1\neq X_2=X_3$ (iv) $X_1\neq X_2\neq X_3$ or (v) $X_1=X_3\neq X_2$, *etc.*

Hypervirulent lineages and bactericidal antibody responses

In general, compositions of the invention are able to induce serum bactericidal antibody responses after being administered to a subject. These responses are conveniently measured in mice and are a standard indicator of vaccine efficacy [*e.g.* see end-note 14 of reference 20]. Serum bactericidal activity (SBA) measures bacterial killing mediated by complement, and can be assayed using human or baby rabbit complement. WHO standards require a vaccine to induce at least a 4-fold rise in SBA in more than 90% of recipients.

Rather than offering narrow protection, compositions of the invention can induce bactericidal antibody responses against more than one serogroup of meningococcus. Within serogroups, compositions may induce antibody responses against more than one hypervirulent lineage. In particular, they can induce bactericidal responses against two or three of the following three hypervirulent lineages: (i) cluster A4; (ii) ET5 complex; and (iii) lineage 3. They may additionally induce bactericidal antibody responses against one or more of hypervirulent lineages subgroup I, subgroup III, subgroup IV-1 or ET-37 complex, and against other lineages *e.g.* hyperinvasive

lineages. However, compositions need not induce bactericidal antibodies against each and every strain of a particular hypervirulent lineage.

Preferred compositions can induce bactericidal responses against: (a) strain 860800, ES13822, ES15085 and/or ES14487 of serogroup Y meningococcus; (b) strain F6124 of serogroup A meningococcus; (c) strain LPN17592 of serogroup W135 meningococcus; (d) strain C11 of serogroup C meningococcus; (e) within serogroup B meningococcus: (i) from cluster A4, strain 961-5945 (B:2b:P1.21,16) and/or strain G2136 (B:–); (ii) from ET-5 complex, strain MC58 (B:15:P1.7,16b) and/or strain 44/76 (B:15:P1.7,16); (iii) from lineage 3, strain 394/98 (B:4:P1.4) and/or strain BZ198 (B:NT:–).

Serogroup Y strain 860800 is seen in row 29 of reference 1, and in reference 57. Serogroup A strain F6124 is seen in references 20, 57 & 58. Serogroup C strain C11 is one of the reference strains disclosed in ref. 59. Serogroup B strains 961-5945 and G2136 are both *Neisseria* MLST reference strains [ids 638 & 1002 in ref. 60]. Strain MC58 is widely available (*e.g.* ATCC BAA-335) and was the strain sequenced in reference 13. Strain 44/76 has been widely used and characterised (*e.g.* ref. 61) and is one of the *Neisseria* MLST reference strains [id 237 in ref. 60; row 32 of Table 2 in ref. 1]. Strain 394/98 was originally isolated in New Zealand in 1998, and there have been several published studies using this strain (*e.g.* refs. 62 & 63). Strain BZ198 is another MLST reference strain [id 409 in ref. 60; row 41 of Table 2 in ref. 1].

Immunogenic compositions and medicaments

Compositions of the invention are immunogenic, and are more preferably vaccine compositions. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic.

The pH of the composition is preferably between 6 and 8, preferably about 7. Stable pH may be maintained by the use of a buffer. Where a composition comprises an aluminium hydroxide salt, it is preferred to use a histidine buffer [64]. The composition may be sterile and/or pyrogen-free. Compositions of the invention may be isotonic with respect to humans.

Compositions may be presented in vials, or they may be presented in ready-filled syringes. The syringes may be supplied with or without needles. A syringe will include a single dose of the composition, whereas a vial may include a single dose or multiple doses. Injectable compositions will usually be liquid solutions or suspensions. As an alternative, they may be presented in solid form (*e.g.* freeze-dried) for solution or suspension in liquid vehicles prior to injection.

Compositions of the invention may be packaged in unit dose form or in multiple dose form. For multiple dose forms, vials are preferred to pre-filled syringes. Effective dosage volumes can be routinely established, but a typical human dose of the composition for injection has a volume of 0.5ml.

Where a composition of the invention is to be prepared extemporaneously prior to use (*e.g.* where a component is presented in lyophilised form) and is presented as a kit, the kit may comprise two vials, or it may comprise one ready-filled syringe and one vial, with the contents of the syringe being used to reactivate the contents of the vial prior to injection.

Immunisation of the invention is in a mammal, preferably in a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant); where the vaccine is for therapeutic use, the human is preferably an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

These uses and methods are preferably for the prevention and/or treatment of a disease caused by a *Neisseria* (*e.g.* meningitis, septicaemia, bacteremia, gonorrhoea *etc.*). The prevention and/or treatment of bacterial or meningococcal meningitis is preferred.

One way of checking efficacy of therapeutic treatment involves monitoring Neisserial infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against administered antigens. Immunogenicity of compositions of the invention can be determined by administering them to test subjects (*e.g.* children 12-16 months age, or animal models [65]) and then determining standard parameters including serum bactericidal antibodies (SBA) and ELISA titres (GMT) of total and high-avidity IgG. These immune responses will generally be determined around 4 weeks after administration of the composition, and compared to values determined before administration of the composition. A SBA increase of at least 4-fold or 8-fold is preferred. Where more than one dose of the composition is administered, more than one post-administration determination may be made.

Preferred compositions of the invention can confer an antibody titre in a patient that is superior to the criterion for seroprotection for each antigenic component for an acceptable percentage of human subjects. Antigens with an associated antibody titre above which a host is considered to be seroconverted against the antigen are well known, and such titres are published by organisations such as WHO. Preferably more than 80% of a statistically significant sample of subjects is seroconverted, more preferably more than 90%, still more preferably more than 93% and most preferably 96-100%.

Compositions will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral, vaginal, topical, transdermal, intranasal, ocular, aural, pulmonary or other mucosal administration. Intramuscular administration to the thigh or the upper arm is preferred. Injection may be via a needle (*e.g.* a hypodermic needle), but needle-free injection may alternatively be used. A typical intramuscular dose is 0.5 ml.

The invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. A primary dose schedule may be followed by a booster dose schedule. Suitable timing between priming doses (*e.g.* between 4-16 weeks), and between priming and boosting, can be routinely determined.

Neisserial infections affect various areas of the body and so the compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition be prepared for oral administration *e.g.* as a tablet or capsule, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as spray, drops, gel or powder [*e.g.* refs 66 & 67]. Success with nasal administration of pneumococcal saccharides [68,69], pneumococcal polypeptides [70], Hib saccharides [71], MenC saccharides [72], and mixtures of Hib and MenC saccharide conjugates [73] has been reported.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials, and a typical quantity of each meningococcal saccharide antigen per dose is between 1μg and 20μg *e.g.* about 1μg, about 2.5μg, about 4μg, about 5μg, or about 10μg (expressed as saccharide).

Further non-antigen components of the composition

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, sucrose [74], trehalose [75], lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. Sterile pyrogen-free, phosphate-

buffered physiologic saline is a typical carrier. A thorough discussion of pharmaceutically acceptable excipients is available in reference 76.

Compositions of the invention may include an antimicrobial, particularly when packaged in multiple dose format.

Compositions of the invention may comprise detergent *e.g.* a Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels *e.g.* <0.01%.

Compositions of the invention may include sodium salts (*e.g.* sodium chloride) to give tonicity. A concentration of 10±2mg/ml NaCl is typical.

Compositions of the invention will generally include a buffer. A phosphate buffer is typical.

Compositions of the invention may comprise a sugar alcohol (*e.g.* mannitol) or a disaccharide (*e.g.* sucrose or trehalose) *e.g.* at around 15-30mg/ml (*e.g.* 25 mg/ml), particularly if they are to be lyophilised or if they include material which has been reconstituted from lyophilised material. The pH of a composition for lyophilisation may be adjusted to around 6.1 prior to lyophilisation.

Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant. Adjuvants which may be used in compositions of the invention include, but are not limited to:

A. Mineral-containing compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulphates, *etc.* [*e.g.* see chapters 8 & 9 of ref. 77], or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt [78].

Aluminium phosphates are particularly preferred, particularly in compositions which include a *H.influenzae* saccharide antigen, and a typical adjuvant is amorphous aluminium hydroxyphosphate with PO_4/Al molar ratio between 0.84 and 0.92, included at 0.6mg Al^{3+}/ml. Adsorption with a low dose of aluminium phosphate may be used *e.g.* between 50 and 100μg Al^{3+} per conjugate per dose. Where there is more than one conjugate in a composition, not all conjugates need to be adsorbed.

B. Oil Emulsions

Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 [Chapter 10 of ref. 77; see also ref. 79] (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

C. Saponin formulations [chapter 22 of ref. 77]

Saponin formulations may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsaprilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. QS21 is marketed as Stimulon™.

Saponin compositions have been purified using HPLC and RP-HPLC. Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in ref. 80. Saponin formulations may also comprise a sterol, such as cholesterol [81].

Combinations of saponins and cholesterols can be used to form unique particles called immunostimulating complexs (ISCOMs) [chapter 23 of ref. 77]. ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of QuilA, QHA & QHC. ISCOMs are further described in refs. 81-83. Optionally, the ISCOMS may be devoid of additional detergent [84].

A review of the development of saponin based adjuvants can be found in refs. 85 & 86.

D. Virosomes and virus-like particles

Virosomes and virus-like particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in refs. 87-92. Virosomes are discussed further in, for example, ref. 93

E. Bacterial or microbial derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof.

Non-toxic derivatives of LPS include monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 de-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in

ref. 94. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22μm membrane [94]. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529 [95,96].

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in refs. 97 & 98.

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a dinucleotide sequence containing an unmethylated cytosine linked by a phosphate bond to a guanosine). Double-stranded RNAs and oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. References 99, 100 and 101 disclose possible analog substitutions *e.g.* replacement of guanosine with 2'-deoxy-7-deazaguanosine. The adjuvant effect of CpG oligonucleotides is further discussed in refs. 102-107.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT [108]. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 109-111. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 108 & 112-114.

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E.coli* (*E.coli* heat labile enterotoxin "LT"), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in ref. 115 and as parenteral adjuvants in ref. 116. The toxin or toxoid is preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LT-G192. The use of ADP-ribosylating toxins and detoxified derivaties thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in refs. 117-124. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in ref. 125, specifically incorporated herein by reference in its entirety.

F. Human immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 [126], *etc.*) [127], interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor.

G. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres [128] or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention [129].

H. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

I. Liposomes (Chapters 13 & 14 of ref. 77)

Examples of liposome formulations suitable for use as adjuvants are described in refs. 130-132.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters [133]. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol [134] as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol [135]. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

K. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in refs. 136 and 137.

L. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

M. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues (*e,g.* "Resiquimod 3M"), described further in refs. 138 and 139.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention: (1) a saponin and an oil-in-water emulsion [140]; (2) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) [141]; (3) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) + a cholesterol; (4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) [142]; (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [143]; (6) SAF, containing 10% squalane, 0.4% Tween 80™, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion. (7) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and (8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dMPL).

Other substances that act as immunostimulating agents are disclosed in chapter 7 of ref. 77.

The use of an aluminium hydroxide or aluminium phosphate adjuvant is particularly preferred, and antigens are generally adsorbed to these salts. Aluminium hydroxide is preferably avoided as an adjuvant if the composition includes a Hib antigen. Where an aluminium phosphate it used and desired not to adsorb an antigen to the adjuvant, this is favoured by including free phosphate ions in solution (*e.g.* by the use of a phosphate buffer). Prevention of adsorption can also be achieved by selecting the correct pH during antigen/adjuvant mixing, an adjuvant with an appropriate point of zero charge, and an appropriate order of mixing for different antigens in a composition [144].

Calcium phosphate is another preferred adjuvant.

Further antigens

Compositions of the invention contain five basic meningococcal protein antigens. They may also include further antigens, although it may contain no meningococcal protein antigens other than the five basic antigens. Further antigens for inclusion may be, for example:

- a saccharide antigen from *Haemophilus influenzae* B.
- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the saccharide disclosed in ref. 5 from serogroup C or the saccharides of ref. 8 (see below).
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* 180, 181 182].
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* 145, 146].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* 146, 147].
- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 3 of ref. 148] *e.g.* the CRM_{197} mutant [*e.g.* 149].
- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 4 of ref. 148].

- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3 [*e.g.* refs. 150 & 151]. Cellular pertussis antigen may be used.
- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 34, 35, 37, 152, *etc.*
- polio antigen(s) [*e.g.* 153, 154] such as OPV or, preferably, IPV.

The composition may comprise one or more of these further antigens. Antigens will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen. It is preferred that the protective efficacy of individual saccharide antigens is not removed by combining them, although actual immunogenicity (*e.g.* ELISA titres) may be reduced.

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens. Such DTP combinations can be used to reconstitute lyophilised conjugates.

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity (see below).

Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means [151]).

As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used [*e.g.* refs. 155 to 163]. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein. Similarly, compositions of the invention may comprise proteins which mimic saccharide antigens *e.g.* mimotopes [164] or anti-idiotype antibodies. These may replace individual saccharide components, or may supplement them. As an example, the vaccine may comprise a peptide mimic of the MenC [165] or the MenA [166] capsular polysaccharide in place of the saccharide itself.

Particularly preferred compositions of the invention include either or both of: (a) a saccharide antigen from *Haemophilus influenzae* type B; and/or (b) an antigen from *Streptococcus pneumoniae*. They may also include saccharide antigens from meningococcus serogroups Y, W135, C and A, except that the saccharide from a given serogroup may be included only where the polypeptide(s) and/or OMVs are not for providing protection against that serogroup.

Haemophilus influenzae type B

Where the composition includes a *H.influenzae* type B antigen, it will typically be a Hib capsular saccharide antigen. Saccharide antigens from *H.influenzae* b are well known.

Advantageously, the Hib saccharide is covalently conjugated to a carrier protein, in order to enhance its immunogenicity, especially in children. The preparation of polysaccharide conjugates in general, and of the Hib capsular polysaccharide in particular, is well documented [*e.g.* references 167 to 175 *etc.*]. The invention may use any suitable Hib conjugate. Suitable carrier proteins are described below, and preferred carriers for Hib saccharides are CRM_{197} ('HbOC'), tetanus toxoid ('PRP-T') and the outer membrane complex of *N.meningitidis* ('PRP-OMP').

The saccharide moiety of the conjugate may be a polysaccharide (*e.g.* full-length polyribosylribitol phosphate (PRP)), but it is preferred to hydrolyse polysaccharides to form oligosaccharides (*e.g.* MW from ~1 to ~5 kDa).

A preferred conjugate comprises a Hib oligosaccharide covalently linked to CRM_{197} via an adipic acid linker [176, 177]. Tetanus toxoid is also a preferred carrier.

Administration of the Hib antigen preferably results in an anti-PRP antibody concentration of $\geq 0.15\mu g/ml$, and more preferably $\geq 1\mu g/ml$.

Compositions of the invention may comprise more than one Hib antigen.

Where a composition includes a Hib saccharide antigen, it is preferred that it does not also include an aluminium hydroxide adjuvant. If the composition includes an aluminium phosphate adjuvant then the Hib antigen may be adsorbed to the adjuvant [178] or it may be non-adsorbed [179].

Hib antigens may be lyophilised *e.g.* together with meningococcal antigens.

Streptococcus pneumoniae

Where the composition includes a *S.pneumoniae* antigen, it will typically be a capsular saccharide antigen which is preferably conjugated to a carrier protein [*e.g.* refs. 180 to 182]. It is preferred to include saccharides from more than one serotype of *S.pneumoniae*. For example, mixtures of polysaccharides from 23 different serotype are widely used, as are conjugate vaccines with polysaccharides from between 5 and 11 different serotypes [183]. For example, PrevNar™ [184] contains antigens from seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) with each saccharide individually conjugated to CRM_{197} by reductive amination, with $2\mu g$ of each saccharide per 0.5ml dose ($4\mu g$ of serotype 6B), and with conjugates adsorbed on an aluminium phosphate adjuvant. Compositions of the invention preferably include at least serotypes 6B, 14, 19F and 23F. Conjugates may be adsorbed onto an aluminium phosphate.

As an alternative to using saccharide antigens from pneumococcus, the composition may include one or more polypeptide antigens. Genome sequences for several strains of pneumococcus are available

[185,186] and can be subjected to reverse vaccinology [187-190] to identify suitable polypeptide antigens [191,192]. For example, the composition may include one or more of the following antigens: PhtA, PhtD, PhtB, PhtE, SpsA, LytB, LytC, LytA, Sp125, Sp101, Sp128, Sp130 and Sp130, as defined in reference 193. The composition may include more than one (*e.g.* 2, 3, 4, 5, 6, 7, 8, 9 10, 11, 12, 13 or 14) of these antigens.

In some embodiments, the composition may include both saccharide and polypeptide antigens from pneumococcus. These may be used in simple admixture, or the pneumococcal saccharide antigen may be conjugated to a pneumococcal protein. Suitable carrier proteins for such embodiments include the antigens listed in the previous paragraph [193].

Pneumococcal antigens may be lyophilised *e.g.* together with meningococcal and/or Hib antigens.

Meningococcus serogroups Y, W135, C and A

As mentioned above, polysaccharide vaccines against serogroups A, C, W135 & Y has been known for many years. These vaccines (MENCEVAX ACWY™ and MENOMUNE™) are based on the organisms' capsular polysaccharides and, although they are effective in adolescents and adults, they give a poor immune response and short duration of protection, and they cannot be used in infants.

In contrast to the unconjugated polysaccharide antigens in these vaccines, the recently-approved serogroup C vaccines (Menjugate™ [4], Meningitec™ and NeisVac-C™) include conjugated saccharides. Menjugate™ and Meningitec™ have oligosaccharide antigens conjugated to a CRM_{197} carrier, whereas NeisVac-C™ uses the complete polysaccharide (de-O-acetylated) conjugated to a tetanus toxoid carrier.

Compositions of the present invention preferably include capsular saccharide antigens from one or more of meningococcus serogroups Y, W135, C and A, wherein the antigens are conjugated to carrier protein(s) and are optionally oligosaccharides. Meningococcal capsular polysaccharides and their conjugates can be prepared as described in references 7 and 8.

A typical quantity of each meningococcal saccharide antigen per dose is between 1μg and 20μg *e.g.* about 1μg, about 2.5μg, about 4μg, about 5μg, or about 10μg (expressed as saccharide).

Where a mixture comprises capsular saccharides from both serogroups A and C, the ratio (w/w) of MenA saccharide:MenC saccharide may be greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Where a mixture comprises capsular saccharides from serogroup Y and one or both of serogroups C and W135, the ratio (w/w) of MenY saccharide:MenW135 saccharide may be greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher) and/or that the ratio (w/w) of MenY saccharide:MenC saccharide may be less than 1 (*e.g.* 1:2, 1:3, 1:4, 1:5, or lower). Preferred ratios (w/w) for saccharides from serogroups A:C:W135:Y are: 1:1:1:1; 1:1:1:2; 2:1:1:1; 4:2:1:1; 8:4:2:1; 4:2:1:2; 8:4:1:2; 4:2:2:1; 2:2:1:1; 4:4:2:1; 2:2:1:2; 4:4:1:2; and 2:2:2:1. Preferred ratios (w/w) for saccharides from serogroups

C:W135:Y are: 1:1:1; 1:1:2; 1:1:1; 2:1:1; 4:2:1; 2:1:2; 4:1:2; 2:2:1; and 2:1:1. Using a substantially equal mass of each saccharide is preferred.

Capsular saccharides will generally be used in the form of oligosaccharides. These are conveniently formed by fragmentation of purified capsular polysaccharide (*e.g.* by hydrolysis), which will usually be followed by purification of the fragments of the desired size.

Fragmentation of polysaccharides is preferably performed to give a final average degree of polymerisation (DP) in the oligosaccharide of less than 30 (*e.g.* between 10 and 20, preferably around 10 for serogroup A; between 15 and 25 for serogroups W135 and Y, preferably around 15-20; between 12 and 22 for serogroup C; *etc.*). DP can conveniently be measured by ion exchange chromatography or by colorimetric assays [194].

If hydrolysis is performed, the hydrolysate will generally be sized in order to remove short-length oligosaccharides [195]. This can be achieved in various ways, such as ultrafiltration followed by ion-exchange chromatography. Oligosaccharides with a degree of polymerisation of less than or equal to about 6 are preferably removed for serogroup A, and those less than around 4 are preferably removed for serogroups W135 and Y.

Preferred MenC saccharide antigens are disclosed in reference 5, as used in Menjugate™.

Saccharides are preferably prepared separately (including any fragmentation, conjugation, modification, *etc.*) and then admixed to give a composition of the invention.

Where the composition comprises capsular saccharide from serogroup A, however, it is preferred that the serogroup A saccharide is not combined with the other saccharide(s) until shortly before use, in order to minimise the potential for hydrolysis. This can conveniently be achieved by having the serogroup A component (typically together with appropriate excipients) in lyophilised form and the other serogroup component(s) in liquid form (also with appropriate excipients), with the liquid components being used to reconstitute the lyophilised MenA component when ready for use. Where an aluminium salt adjuvant is used, it is preferred to include the adjuvant in the vial containing the with the liquid vaccine, and to lyophilise the MenA component without adjuvant. A composition of the invention may thus be prepared from a kit comprising: (a) capsular saccharide from *N.meningitidis* serogroup A, in lyophilised form; and (b) the further antigens from the composition, in liquid form.

Covalent conjugation

Capsular saccharides in compositions of the invention will usually be conjugated to carrier protein(s). In general, conjugation enhances the immunogenicity of saccharides as it converts them from T-independent antigens to T-dependent antigens, thus allowing priming for immunological memory. Conjugation is particularly useful for paediatric vaccines and is a well known technique [*e.g.* reviewed in refs. 196 and 167-175].

Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria toxoid or tetanus toxoid. The CRM_{197} diphtheria toxin mutant [197-199] is particularly preferred. Other suitable carrier proteins include the *N.meningitidis* outer membrane protein [200], synthetic peptides [201,202], heat shock proteins [203,204], pertussis proteins [205,206], cytokines [207], lymphokines [207], hormones [207], growth factors [207], artificial proteins comprising multiple human $CD4^+$ T cell epitopes from various pathogen-derived antigens [208], protein D from *H.influenzae* [209,210], pneumococcal surface protein PspA [211],iron-uptake proteins [212], toxin A or B from *C.difficile* [213], *etc.* Preferred carriers are diphtheria toxoid, tetanus toxoid, *H.influenzae* protein D, and CRM_{197}.

Within a composition of the invention, it is possible to use more than one carrier protein *e.g.* to reduce the risk of carrier suppression. Thus different carrier proteins can be used for different serogroups *e.g.* serogroup A saccharides might be conjugated to CRM_{197} while serogroup C saccharides might be conjugated to tetanus toxoid. It is also possible to use more than one carrier protein for a particular saccharide antigen *e.g.* serogroup A saccharides might be in two groups, with some conjugated to CRM_{197} and others conjugated to tetanus toxoid. In general, however, it is preferred to use the same carrier protein for all saccharides.

A single carrier protein might carry more than one saccharide antigen [214]. For example, a single carrier protein might have conjugated to it saccharides from serogroups A and C. To achieve this goal, saccharides can be mixed prior to the conjugation reaction. In general, however, it is preferred to have separate conjugates for each serogroup.

Conjugates with a saccharide:protein ratio (w/w) of between 1:5 (*i.e.* excess protein) and 5:1 (*i.e.* excess saccharide) are preferred. Ratios between 1:2 and 5:1 are preferred, as are ratios between 1:1.25 and 1:2.5 are more preferred. Excess carrier protein may be preferred for MenA and MenC.

Conjugates may be used in conjunction with free carrier protein [215]. When a given carrier protein is present in both free and conjugated form in a composition of the invention, the unconjugated form is preferably no more than 5% of the total amount of the carrier protein in the composition as a whole, and more preferably present at less than 2% by weight.

Any suitable conjugation reaction can be used, with any suitable linker where necessary.

The saccharide will typically be activated or functionalised prior to conjugation. Activation may involve, for example, cyanylating reagents such as CDAP (*e.g.* 1-cyano-4-dimethylamino pyridinium tetrafluoroborate [216,217,*etc.*]). Other suitable techniques use carbodiimides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, S-NHS, EDC, TSTU; see also the introduction to reference 173).

Linkages via a linker group may be made using any known procedure, for example, the procedures described in references 218 and 219. One type of linkage involves reductive amination of the

polysaccharide, coupling the resulting amino group with one end of an adipic acid linker group, and then coupling a protein to the other end of the adipic acid linker group [171,220,221]. Other linkers include B-propionamido [222], nitrophenyl-ethylamine [223], haloacyl halides [224], glycosidic linkages [225], 6-aminocaproic acid [226], ADH [227], C_4 to C_{12} moieties [228] *etc.* As an alternative to using a linker, direct linkage can be used. Direct linkages to the protein may comprise oxidation of the polysaccharide followed by reductive amination with the protein, as described in, for example, references 229 and 230.

A process involving the introduction of amino groups into the saccharide (*e.g.* by replacing terminal =O groups with $-NH_2$) followed by derivatisation with an adipic diester (*e.g.* adipic acid N-hydroxysuccinimido diester) and reaction with carrier protein is preferred. Another preferred reaction uses CDAP activation with a protein D carrier *e.g.* for MenA or MenC.

After conjugation, free and conjugated saccharides can be separated. There are many suitable methods, including hydrophobic chromatography, tangential ultrafiltration, diafiltration *etc.* [see also refs. 231 & 232, *etc.*].

Where the composition of the invention includes a conjugated oligosaccharide, it is preferred that oligosaccharide preparation precedes conjugation.

As an alternative to purification, capsular saccharides may be obtained by total or partial synthesis *e.g.* Hib synthesis is disclosed in ref. 233, and MenA synthesis in ref. 234.

Further and alternative serogroup B polypeptide antigens

The invention uses a composition which, after administration to a subject, is able to induce an antibody response in that subject, wherein the antibody response is protective against at least serogroup Y of meningococcus. Although NadA, 741, 936, 953 and 287 are preferred antigens for achieving this protection, other MenB polypeptide antigens which may be included in compositions of the invention (optionally in combination with one or more of the five basic antigens) include those comprising one of the following amino acid sequences: SEQ ID NO:650 from ref. 15; SEQ ID NO:878 from ref. 15; SEQ ID NO:884 from ref. 15; SEQ ID NO:4 from ref. 16; SEQ ID NO:598 from ref. 17; SEQ ID NO:818 from ref. 17; SEQ ID NO:864 from ref. 17; SEQ ID NO:866 from ref. 17; SEQ ID NO:1196 from ref. 17; SEQ ID NO:1272 from ref. 17; SEQ ID NO:1274 from ref. 17; SEQ ID NO:1640 from ref. 17; SEQ ID NO:1788 from ref. 17; SEQ ID NO:2288 from ref. 17; SEQ ID NO:2466 from ref. 17; SEQ ID NO:2554 from ref. 17; SEQ ID NO:2576 from ref. 17; SEQ ID NO:2606 from ref. 17; SEQ ID NO:2608 from ref. 17; SEQ ID NO:2616 from ref. 17; SEQ ID NO:2668 from ref. 17; SEQ ID NO:2780 from ref. 17; SEQ ID NO:2932 from ref. 17; SEQ ID NO:2958 from ref. 17; SEQ ID NO:2970 from ref. 17; SEQ ID NO:2988 from ref. 17, or a polypeptide comprising an amino acid sequence which: (a) has 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to said sequences; and/or (b) comprises a fragment of at least *n*

consecutive amino acids from said sequences, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments for (b) comprise an epitope from the relevant sequence. More than one (*e.g.* 2, 3, 4, 5, 6) of these polypeptides may be included.

The antigens transferrin binding protein and/or Hsf protein may also be used [235]. The NspA protein can also be used [236], preferably recombinantly expressed and purified as in reference 237.

General

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 238. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in reference 239.

The term "polypeptide" generally refers to a polymer of amino acid residues, and is not limited to a minimum length of the product. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. Typically, polypeptides useful in this invention can have a maximum length suitable for the intended application. Generally, the maximum length is not critical and can easily be selected by one skilled in the art.

Polypeptides of the invention can be prepared in many ways *e.g.* by chemical synthesis (at least in part), by digesting longer polypeptides using proteases, by translation from RNA, by purification from cell culture (*e.g.* from recombinant expression), from the organism itself (*e.g.* after bacterial culture), from a cell line source *etc.* A preferred method for production of peptides <40 amino acids long involves *in vitro* chemical synthesis [240,241]. Solid-phase peptide synthesis is particularly preferred, such as methods based on tBoc or Fmoc [242] chemistry. Enzymatic synthesis [243] may also be used in part or in full. As an alternative to chemical synthesis, biological synthesis may be used *e.g.* the polypeptides may be produced by translation. This may be carried out *in vitro* or *in vivo*. Biological methods are in general restricted to the production of polypeptides based on L-amino

acids, but manipulation of translation machinery (*e.g.* of aminoacyl tRNA molecules) can be used to allow the introduction of D-amino acids (or of other non natural amino acids, such as iodotyrosine or methylphenylalanine, azidohomoalanine, etc.) [244]. Where D-amino acids are included, however, it is preferred to use chemical synthesis. Polypeptides of the invention may have covalent modifications at the C-terminus and/or N-terminus.

Polypeptides of the invention can take various forms (*e.g.* native, fusions, glycosylated, non-glycosylated, lipidated, non-lipidated, phosphorylated, non-phosphorylated, myristoylated, non-myristoylated, monomeric, multimeric, particulate, denatured, *etc.*). A purified polypeptide is separate and discrete from the whole organism in which is was expressed.

The term "nucleic acid" includes in general means a polymeric form of nucleotides of any length, which contain deoxyribonucleotides, ribonucleotides, and/or their analogs. It includes DNA, RNA, DNA/RNA hybrids. It also includes DNA or RNA analogs, such as those containing modified backbones (*e.g.* peptide nucleic acids (PNAs) or phosphorothioates) or modified bases. Thus the invention includes mRNA, tRNA, rRNA, ribozymes, DNA, cDNA, recombinant nucleic acids, branched nucleic acids, plasmids, vectors, probes, primers, *etc.*. Where nucleic acid of the invention takes the form of RNA, it may or may not have a 5' cap.

Nucleic acids of the invention can be prepared in many ways *e.g.* by chemical synthesis (at least in part), by digesting longer nucleic acids using nucleases (*e.g.* restriction enzymes), by joining shorter nucleic acids (*e.g.* using ligases or polymerases), from genomic or cDNA libraries, *etc.*

Sequences included in nucleic acids and polypeptides to facilitate cloning or purification, *etc.*, do not necessarily contribute to the invention and may be omitted or removed.

MODES FOR CARRYING OUT THE INVENTION

Polypeptides

ΔG287-953 hybrid polypeptide, 936-ΔG741 hybrid polypeptide and $NadA^{(NL)(C)}$ polypeptide were prepared as disclosed in reference 24. These polypeptides are encoded by sequences taken from the genomes of serogroup B strains of meningococcus.

The three polypeptides were mixed to give a combined formulation, including an aluminium hydroxide adjuvant. The formulation was used to immunise mice, and bactericidal titres of immune sera were assessed against meningococcal strains in serogroups A, B, C, W135 and Y. Results against 11 strains were as follows:

Serogroup	B				A	C	W135	Y			
Strain	2996	MC58	394/98	44/76	F6124	C11	LPN17592	860800	ES13822	ES15085	ES14487
SBA titre	1024	4096	1024	8192	2048	2048	512	65536	4096	4096	4096

Thus the mixed composition was effective in raising sera that were bactericidal against serogroup B, which is the serogroup of origin for the amino acid sequences included in the polypeptide. Titres in the same range were seen against serogroups A and C, and slightly lower titres were seen against serogroup W. Surprisingly, the highest titres were seen against strains in serogroup Y.

Moreover, the titres seen against the serogroup Y strains were equivalent to those obtained using a tetravalent A/C/W135/Y conjugate vaccine [8]:

Strain	860800	ES13822	ES15085	ES14487
Polypeptides	65536	4096	4096	4096
Tetravalent conjugates	32768	>8192	>8192	4096

Thus, for the first time, the inventors have achieved effective immune responses against meningococcal strains from each of the pathogenic serogroups (A, B, C, W135 and Y) using polypeptide antigens and without using capsular saccharides.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. A method of immunising a subject against infection by serogroup Y of *Neisseria meningitidis*, comprising administering to the subject a composition comprising one or more immunogenic polypeptides.

2. A method of immunising a subject against infection by serogroup Y of *Neisseria meningitidis*, comprising administering to the subject a composition comprising meningococcal OMVs.

3. The use of one or more immunogenic polypeptides in the manufacture of a medicament for immunising a subject against infection by serogroup Y of *Neisseria meningitidis*.

4. The use of meningococcal OMVs in the manufacture of a medicament for immunising a subject against infection by serogroup Y of *Neisseria meningitidis*.

5. The method or use of any one of claims 1 to 4, for immunising a subject also against infection by serogroup A, B, C and/or W135 or *N.meningitidis*.

SEQUENCE LISTING

SEQ ID NO: 1 – NadA from strain 2996, with C-terminus deletion

MKHFPSKVLTTAILATFCSGALAATNDDDVKKAATVAIAAAYNNGQEINGFKAGETIYDIDEDGTITKKDATAADVEADDF
KGLGLKKVVTNLTKTVNENKQNVDAKVKAAESEIEKLTTKLADTDAALADTDAALDATTNALNKLGENITTFAEETKTNIV
KIDEKLEAVADTVDKHAEAFNDIADSLDETNTKADEAVKTANEAKQTAEETKQNVDAKVKAAETAAGKAEAAAGTANTAAD
KAEAVAAKVTDIKADIATNKDNIAKKANSADVYTREESDSKFVRIDGLNATTEKLDTRLASAEKSIADHDTRLNGLDKTVS
DLRKETRQGLAEQAALSGLFQPYNVG

SEQ ID NO: 2 – NadA from strain 2996, with C-terminus deletion and leader peptide processed

ATNDDDVKKAATVAIAAAYNNGQEINGFKAGETIYDIDEDGTITKKDATAADVEADDFKGLGLKKVVTNLTKTVNENKQNV
DAKVKAAESEIEKLTTKLADTDAALADTDAALDATTNALNKLGENITTFAEETKTNIVKIDEKLEAVADTVDKHAEAFNDI
ADSLDETNTKADEAVKTANEAKQTAEETKQNVDAKVKAAETAAGKAEAAAGTANTAADKAEAVAAKVTDIKADIATNKDNI
AKKANSADVYTREESDSKFVRIDGLNATTEKLDTRLASAEKSIADHDTRLNGLDKTVSDLRKETRQGLAEQAALSGLFQPY
NVG

SEQ ID NO: 3 – ΔG741 from MC58 strain

VAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQL
ITLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYT
IDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEVKTVNGIRHIG
LAAKQ

SEQ ID NO: 4 – 936 from MC58 strain with leader peptide processed

VSAVIGSAAVGAKSAVDRRTTGAQTDDNVMALRIETTARSYLRQNNQTKGYTPQISVVGYNRHLLLLGQVATEGEKQFVGQ
IARSEQAAEGVYNYITVASLPRTAGDIAGDTWNTSKVRATLLGISPATQARVKIVTYGNVTYVMGILTPEEQAQITQKVST
TVGVQKVITLYQNYVQR

SEQ ID NO: 5 – 953 from MC58 strain with leader peptide processed

ATYKVDEYHANARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIPIANLQSGSQHFTDHLKSADIFDAAQYPDIR
FVSTKFNFNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPMEKTEVCGGDFSTTIDRTKWGMDYLVNVGMTKSVRIDIQ
IEAAKQ

SEQ ID NO: 6 – ΔG287 from MC58 strain

SPDVKSADTLSKPAAPVVSEKETEAKEDAPQAGSQGQGAPSAQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQNDMPQN
AAGTDSSTPNHTPDPNMLAGNMENQATDAGESSQPANQPDMANAADGMQGDDPSAGGQNAGNTAAQGANQAGNNQAAGSSD
PIPASNPAPANGGSNFGRVDLANGVLIDGPSQNITLTHCKGDSCSGNNFLDEEVQLKSEFEKLSDADKISNYKKDGKNDKF
VGLVADSVQMKGINQYIIFYKPKPTSFARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYL
TYGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGSKSVDGIIDSGDDLHMGTQKF
KAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQD

SEQ ID NO: 7 – 287-953 hybrid

MASPDVKSADTLSKPAAPVVSEKETEAKEDAPQAGSQGQGAPSAQGGQDMAAVSEENTGNGGAAATDKPKNEDEGAQNDMP
QNAADTDSLTPNHTPASNMPAGNMENQAPDAGESEQPANQPDMANTADGMQGDDPSAGGENAGNTAAQGTNQAENNQTAGS
QNPASSTNPSATNSGGDFGRTNVGNSVVIDGPSQNITLTHCKGDSCSGNNFLDEEVQLKSEFEKLSDADKISNYKKDGKND
GKNDKFVGLVADSVQMKGINQYIIFYKPKPTSFARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPE
GNYRYLTYGAEKLPGGSYALRVQGEPSKGEMLAGTAVYNGEVLHFHTENGRPSPSRGRFAAKVDFGSKSVDGIIDSGDGLH
MGTQKFKAAIDGNGFKGTWTENGGGDVSGKFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQDGSGGGGATYKVDEYHA
NARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIPVANLQSGSQHFTDHLKSADIFDAAQYPDIRFVSTKFNFNG
KKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDRTKWGVDYLVNVGMTKSVRIDIQIEAAKQ*

SEQ ID NO: 8 – 936-741 hybrid

MVSAVIGSAAVGAKSAVDRRTTGAQTDDNVMALRIETTARSYLRQNNQTKGYTPQISVVGYNRHLLLLGQVATEGEKQFVG
QIARSEQAAEGVYNYITVASLPRTAGDIAGDTWNTSKVRATLLGISPATQARVKIVTYGNVTYVMGILTPEEQAQITQKVS

TTVGVQKVITLYQNYVQRGSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLN
TGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLP
EGGRATYRGTAFGSDDAGGKLTYTIDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGI
FGGKAQEVAGSAEVKTVNGIRHIGLAAKQ*

SEQ ID NO: 9 – linker

GSGGGG

SEQ ID NO: 10 – 741 sequence

CSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQ
IEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDA
GGKLTYTIDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEVKTV
NGIRHIGLAAKQ

SEQ ID NO: 11 – 741 sequence

CSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQ
IEVDGQLITLESGEFQIYKQDHSAVVALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDAG
GKLTYTIDFAAKQGHGKIEHLKTPEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHLALFGDRAQEIAGSATVKIGE
KVHEIGIAGKQ

SEQ ID NO: 12 – 741 sequence

CSSGGGGSGGGGVAADIGTGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLSAQGAEKTFKAGDKDNSLNTGKLKNDKI
SRFDFVQKIEVDGQTITLASGEFQIYKQNHSAVVALQIEKINNPDKTDSLINQRSFLVSGLGGEHTAFNQLPGGKAEYHGK
AFSSDDPNGRLHYSIDFTKKQGYGRIEHLKTLEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHLALFGDRAQEIAG
SATVKIGEKVHEIGIAGKQ